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AUTHOR'S VIEWS

Epigenetic condensates regulate chromatin activity and tumorigenesis

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ABSTRACT

Alterations of epigenetic modulators are extensively associated with cancer, but their key molecular activities in cancer regulation are often unclear. We discovered that lysine demethylase 6A (KDM6A, also known as UTX) suppresses cancer by forming liquid-like condensates with lysine methyltransferase 2D (KMT2D, also known as MLL4) and regulating chromatin activity at multiple levels.

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Epigenetic modulators play critical roles in development and their alterations are extensively associated with diseases including cancer. However, their key molecular properties in development and disease control are often not fully understood, posing a major barrier to further understanding and targeting these modulators. For example, it is not uncommon that the catalytic activities of some of these epigenetic enzymes are dispensable for their biological or pathological roles. Lysine demethylase 6A (KDM6A, also known as UTX) is such an epigenetic enzyme. It is a histone H3K27 demethylase associated with the lysine methyltransferase 2 C (KMT2C, also known as MLL3) and lysine methyltransferase 2D (KMT2D, also known as MLL4) complexes that are H3K4 mono-methylases and also histone acetyltransferase E1A binding protein p300 (EP300, also known as p300), all mainly at enhancers.[1] *UTX* as well as *MLL3* and *MLL4* are important tumor suppressors that are among the most frequently mutated chromatin regulator genes across a broad spectrum of human cancer.[2,3] UTX and MLL4 also play important roles in stem cell differentiation and multiple developmental pathways, and their mutations are the primary cause of Kabuki syndrome, a neurodevelopmental disorder. Intriguingly, the demethylase activity of UTX is often dispensable in regulating stem cell differentiation, development, and cancer suppression.[1] The key molecular properties of UTX in regulating chromatin in cancer and development remain unclear.

Our recent work shows that UTX undergoes liquid–liquid phase separation that is mediated by its core Intrinsically Disordered Region (cIDR), and this property is critical for its tumor suppressive activity in leukemia and pancreatic cancer models.[4] We show that the cIDR is lost in the most frequent UTX mutation in all cancers, and it is the loss of cIDR, not the region after IDR (including the catalytic region), that is responsible for UTX inactivation as a tumor suppressor in these patients. UTX's tumor suppressive activity is abolished by mutagenesis in cIDR, but can be regained in chimeric proteins that substitute the cIDR with a condensation-capable IDR from irrelevant proteins. Moreover, following genomic editing, we showed that fluorescently tagged endogenous UTX forms dynamic nuclear condensates in embryonic stem cells, and both the condensation and stem cell differentiation are impaired upon loss of the endogenous IDR. These data establish an important role of phase separation for UTX in tumor suppression as well as stem cell function.

We then delved into the molecular mechanisms using in vitro reconstitution and biochemical assays, a number of engineered cell systems, and genome-wide approaches. Our data show that, through direct interaction of specific domains on UTX and MLL4, UTX condenses MLL4 into the same droplets on chromatin and greatly enhances its H3K4 mono-methylation activity (Figure 1). UTX also co-condenses p300 into specific compartments as well. Forming condensates is also required for the H3K27 demethylation activity of UTX in cells, and is thus a fundamental property of UTX for its tumor suppressive activity regardless whether H3K27 demethylation is required for tumor suppression or not.

In line with UTX acting as both a gene activator and repressor,[5] we show that UTX both promotes and represses various histone modifications and long-range chromatin interactions at different genomic regions. Importantly, the different effects of UTX on chromatin all require its condensation property, as the diffused UTX mutants not only failed to achieve efficient histone modifications and chromatin interactions at the genomic sites normally promoted by UTX condensates, but also resulted in aberrant chromatin modifications and looping at sites normally suppressed by UTX condensates. Therefore, UTX condensation ensures efficient and correct chromatin modifications and high-order organization to orchestrate a proper tumor-suppressive transcriptional program (Figure 1).[4]

UTX is located on X chromosome but escapes X–inactivation, and this kind of tumor suppressors were recently found to substantially contribute to the lower cancer incidence in females.[6] The tumor suppressive activity of UTY, the Y chromosome homolog of UTX, is considerably weaker than

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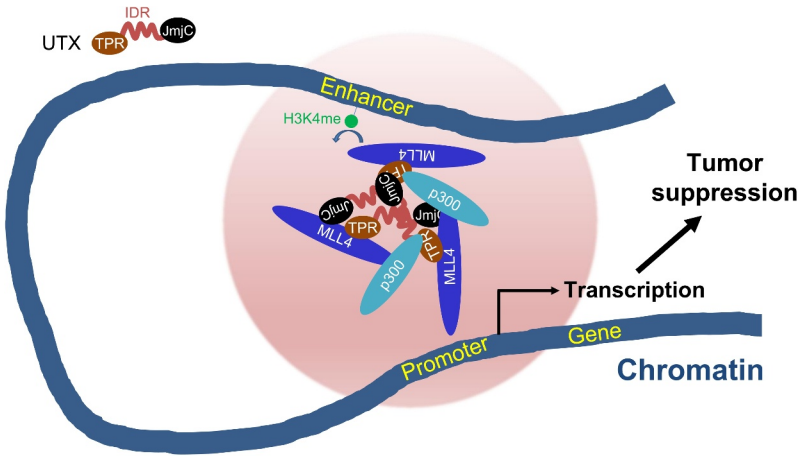

Figure 1. Regulation of chromatin activity by UTX condensation for tumor suppression. The tumor suppressor protein lysine demethylase 6A (KDM6A, also known as UTX) contains the N-terminal tetratricopeptide repeat (TPR) domain, the central intrinsically disordered region (IDR), and the C-terminal Jumonji C (JmjC) domain. UTX associates with lysine methyltransferase 2D (KMT2D, also known as MLL4) and E1A binding protein p300 (EP300, also known as p300) through its TPR domain, and forms co-condensates with these proteins in a IDR-dependent manner. These condensates concentrate the activity of these epigenetic regulators on chromatin including facilitating the H3K4 methylation (H3K4me) activity of MLL4 on gene enhancers, and also regulate chromatin interactions between gene promoter and enhancer. UTX condensation thus underlies its activity in chromatin regulation to orchestrate a transcriptional program that is important for tumor suppression.

UTX.[5,6] In the last part of our work, we found that cIDR is the key determinant of the lower tumor suppressive activity of UTY, as replacing the cIDR of UTX by that of UTY significantly reduced the tumor suppressive effect. We then found that UTY cIDR has different sequence features from UTX cIDR, and has a stronger propensity than UTX cIDR to form condensates. However, UTY condensates are less dynamic and diffuse more slowly than UTX condensates. The slower dynamics of UTY probably reduces the chromatin-modulatory reaction rate in the condensates and renders UTY a weaker tumor suppressor than UTX. Indeed, our engineered UTX mutant that substitutes all of its cIDR histidine residues with tyrosine (known to promote phase separation) also has stronger condensation ability but the condensates are less dynamic. Consequently, this mutant also has reduced tumor-suppressive activity.[4] These findings join our previous studies in showing that the biophysical properties of protein condensates are important for a gene regulator in controlling tumorigenesis.[7,8]

In summary, our study reveals a fundamental activity of UTX in tumor suppression, and mechanistically integrates its activity with that of MLL4, another important player in chromatin and cancer biology. UTX phase separation is also important for stem cell differentiation and most likely underlie its roles in development and Kabuki syndrome. Phase separation, as a fundamental principle in organizing cellular space and biochemistry,[9,10] may also be important for other epigenetic modulators involved in cancer, considering the widespread IDRs in these proteins. Moreover, it helps establish a new concept that key variations in a fundamental principle of cellular organization may contribute to the sexual dimorphisms in cancer incidence.

Disclosure statement

No potential conflict of interest were disclosed.

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